

Amendments to the Specification

Please replace the table entitled Table 4 that begins at page 46 and ends on page 47 with the following redlined Table 4.

Table 4. List of PCR oligonucleotide primers designed for the generation of modified nspA genes listed in Table 2.

Primer	Sequence 5' - 3'	Restriction site
DMAR839	ggaattccatatgaaaaagcacttgccac (SEQ ID NO:3)	NdeI
DMAR840	ataagaatgcggccgctcagaattgacgcgcac (SEQ ID NO:4)	NotI
DMAR937	tcgaggtacccgtgtaatcgacggcgaagc (SEQ ID NO:5)	KpnI
DMAR938	tcgaggtaccctttacagcatcgggcgcg (SEQ ID NO:6)	KpnI
DMAR1149	tcgaggtacctgttttgcgtgtcgggcatcgg (SEQ ID NO:7)	KpnI
DMAR1152	tcgaggtaccaaaggcttcagcccgcgc (SEQ ID NO:8)	KpnI
DMAR1153	atatgggcccggcgcggttgaggctcaagc (SEQ ID NO:9)	ApaI
DMAR1154	atatgggccctccaacacctccatcggcctcggcg (SEQ ID NO:10)	ApaI
DMAR1157	cgataatggcgaactgtccgtcggcgtgcgcgtcaaattctgagc (SEQ ID NO:11)	-
DMAR1158	ggccgctcagaatttgacgcgcacgccgacggacagttcgccattatcgggcc (SEQ ID NO:12)	-
DMAR1159	atatgggcccgtagttgtagcggtagccggc (SEQ ID NO:13)	ApaI
DMAR1160	tcgaggtacccgtgtaatcgacggcgaagcg (SEQ ID NO:14)	KpnI
DMAR1161	tcgaggtaccctttacagcatcgggcgcgtcc (SEQ ID NO:15)	KpnI

Please replace the table entitled Table 5 on page 46 with the following redlined Table 5.

Table 5. List of PCR oligonucleotide primer sets used for site-directed mutagenesis on modified nspA genes

Gene/Protein designation	Primer Identification	Primer SEQUENCE 5' ---> 3'
Nm3	DMAR837	ccgcgctccgctcgacttgccggcagcaacagcttcagccaaac (SEQ ID NO:16)
	DMAR838	gtttggctgaagctgtcgctgccgccaagtgcacggaggcgcg (SEQ ID NO:17)
Nm14	DMAR941	cgcttcgccgtcgattacacgggtaacctttacagcatcggcgcg (SEQ ID NO:18)
	DMAR942	cgcgccgatgctgtaaagggtaccctgtaatcgacggcgaagcg (SEQ ID NO:19)
Nm16	DMAR1150	gcgcgggctgaagcctttgttacctgttttgcgtgtcggc (SEQ ID NO:20)
	DMAR1151	gccgcacacgcaaaaacaggtacaaaaggcttcagcccgcgc (SEQ ID NO:21)
Nm17	DMAR1155	ttgagcctcaaccgcgccgggggtccaacacctccatcggcctc (SEQ ID NO:22)
	DMAR1156	gaggccgatggaggtgttgagccccggcgcggttgaggctcaa (SEQ ID NO:23)
Nm20	DMAR1162	ggacgcgccgatgctgtaaagggtaccctgtaatcgacggcgaa (SEQ ID NO:24)
	DMAR1163	ttcgccgtcgattacacgggtaacctttacagcatcggcgcgtcc (SEQ ID NO:25)

Please replace the table entitled Table 6 that begins at page 47 and ends on page 48 with the following redlined Table 6.

Table 6. List of modifications on modified nspA gene products generated by site-directed mutagenesis

Gene/Protein designation	Molecule used for mutagenesis	DNA modifications ¹
Nm3	<u>nspA</u>	341-TGG <u>C</u> CGGCAGCA <u>A</u> CA-355 (SEQ ID NO:26)
Nm14	<u>Nm14</u>	201-GGGTAACCTT-210 (SEQ ID NO:27)
Nm16	<u>Nm16</u>	111-TAA <u>C</u> AAAGGC-120 (SEQ ID NO:28)
Nm17	<u>Nm17</u>	331-GGGGGCTCCA-340 (SEQ ID NO:29)
Nm20	<u>Nm20</u>	181-ACGGGTA <u>A</u> CC-190 (SEQ ID NO:30)

¹ The underlined amino acid residues represent the modification in DNA sequence.

Please replace the paragraph that begins at page 48, line 15 with the following redlined paragraph.

The epitopes recognized by group III MAbs, such as Me-16, were easily located using overlapping 15- to 20-amino-acid- residue synthetic peptides covering the full-length of the NspA protein. These peptides were presented in the patent PCT/WO/96/29412. As an example, MAb Me-16 was found by ELISA to react with two separate peptides located between residues 41-55 (GSAKGFSPRISAGYR) (SEQ ID NO:31) and 141-150 (VDLDAGYRYNYIGKV) (SEQ ID NO:32). Closer analysis revealed that these two peptides shared the AGYR residues, which are underlined in the peptide sequences. According to the NspA model (Figure 2), these two regions are embedded inside the meningococcal outer membrane and as expected, antibodies directed against these regions did not attach to intact meningococcal cells (Figure 3).

Please replace the paragraph that begins at page 53, line 19 with the following redlined paragraph.

Liposomes ~~were~~are prepared using a dialysis method. Liposomes ~~were~~are prepared with different synthetic (see list 1 in this Example) or bacterial phospholipids with or without cholesterol, which ~~were~~are combined at different ratios. Some liposome formulations ~~were~~are also prepared with the adjuvant monophosphoryl lipid A (MPLA, Avanti polar lipids, Alabaster, AL) at 600 $\mu\text{g/ml}$. NspA protein ~~was~~is first precipitated in 99% ethanol (vol/vol) and denatured in 1 ml of PBS buffer containing 1% (wt/vol) of SDS (Sigma chemical), and heated at 100° C. for 10 min. The solution ~~was~~is diluted with 1 ml of PBS buffer containing 15% (wt/vol) of n-octyl β -D-glucopyranoside (OG, Sigma) and incubated at room temperature for 3 h. Lipids ~~were~~are dissolved in a chloroform:methanol solution (2:1) in a round bottom glass flask and dried using a rotatory evaporator (Rotavapor, Büchi, Switzerland) to achieve an even film on the vessel. The above protein-detergent solution ~~was~~is then added to the lipid film and mixed gently until the film ~~was~~is dissolved. The solution, after mixing, ~~was~~is slightly opalescent in appearance. The solution ~~was~~is then extensively dialysed against PBS buffer (pH 7.4) to remove detergent and to induce liposome formation. After dialysis, the resulting milky solution ~~was~~is sequentially extruded through 1000, 400, 200, and 100 nm polycarbonate filters using a stainless steel extrusion device (Lipex Biomembranes, Vancouver, Canada). The recombinant NspA not incorporated into the liposome ~~was~~is removed by centrifugation at 20000 g for 15 min at 4° C. The liposome solution was centrifuged at 250000 g for 1 h at 4° C and the pellet ~~was~~is suspended with PBS buffer containing 0.3 M of sucrose. Vesicle size and homogeneity ~~were~~are evaluated by quasi-elastic light scattering with a submicron particles analyzer (model N4 Plus, Beckman Coulter). Using this apparatus, it ~~was~~is estimated that the liposome size in the different preparations ~~was~~is approximately 100 nm. All liposome preparations ~~were~~are sterilized by filtration through a 0.22 μm membrane and stored at -80° C until used. The amount of recombinant protein incorporated in the liposome ~~was~~is estimated by MicroBCA (Pierce, Rockford, Ill.) after protein extraction of NspA-liposome preparations with chloroform:methanol solution (2:1) as described by Wessel and Flügge (Anal. Biochem. 1984, 138:141-143).

Please replace the paragraph that begins at page 54, line 24 with the following redlined paragraph.

Gel filtration and rapid dilution ~~were~~are used as alternate methods to induce the formation of NspA liposome. For the gel filtration method, the NspA-OG-SDS-lipids solution ~~was~~is applied directly on top of a Sephadex G-50 (column size: 2 × 20cm, Pharmacia) or a P-6 (column size: 2 × 20cm, Bio Rad) size exclusion chromatography/desalting column and eluted with PBS buffer at a flow rate of 2.5 ml/min. Fractions containing both protein and lipids ~~were~~are pooled, extruded, centrifuged, and the vesicle sizes ~~were~~are evaluated as described above. All preparations ~~were~~are sterilized through a 0.22 μ m membrane and stored at -80° C until used.

Please replace the paragraph that begins at page 55, line 4 with the following redlined paragraph.

For rapid dilution method, a lipid film ~~was~~is prepared in a round bottom glass flask as described above. This lipid film ~~was~~is dissolved with a phosphate buffered solution (10 mM, 70 mM NaCl, pH 7.2) containing 1% triton X-100 and 750 μ g/ml of NspA protein. Lipid-detergent-protein solution ~~was~~is then diluted drop-wise (1 drop/sec), with constant stirring, by the addition of 11 volumes of phosphate buffer. After dilution, the solution ~~was~~is kept at room temperature for 30 min with agitation. The recombinant NspA not incorporated into the liposome ~~was~~is removed by centrifugation and the liposome solution ~~was~~is ultracentrifuged as described above. Finally, the liposome pellet ~~was~~is suspended with PBS buffer containing 0.3 M sucrose. Vesicle size and homogeneity ~~were~~are evaluated as described above. All preparations ~~were~~are sterilized through a 0.22 μ m membrane and stored at -80° C until used.